Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 28, SEC. F. SEPTEMBER, 1950 NUMBER 9

THE THIAMINE AND RIBOFLAVIN CONTENT OF MANITOBA GROWN WHEAT, OATS, AND BARLEY OF THE 1947 CROP[1]

BY ALLEN D. ROBINSON,[2] CYRIL H. TOBIAS,[3] AND BARBARA J. MILES[3]

Abstract

The averages and ranges for thiamine in approximately 250 samples of cereals tested, expressed as μgm. per gm., were: barley 4.2 (3.3–5.7), oats 6.7 (3.8–8.6), wheat 4.5 (3.4–5.9); for riboflavin: barley 1.3 (0.9–1.6), oats 1.3 (1.0–1.7), wheat 1.2 (1.0–1.4). There was a marked increase in the thiamine content of oats over that for 1946—amounting to 0.7 μgm. per gm. Barley and wheat showed slight increases. The values for riboflavin in the three cereals grown in 1947 were about the same as those for the 1946 samples. Varietal effects on thiamine content differed somewhat from those observed in 1946. For barley, Garton, OAC21, Sanalta, and Montcalm were about equal and slightly better than Plush. The order of thiamine content of wheat—for those varieties of which more than 10 samples were tested—was Carlton, Regent, Redman, Thatcher, and agreed with the findings for the previous year. Durum wheats showed higher thiamine levels than spring wheats. There was no varietal effect on the thiamine content of oats. There was no varietal effect on riboflavin content of any of the cereals. None was observed for the 1946 samples. For 1946 no soil zone effect on vitamin content of any of the cereals was noted. In 1947 rendzina and black earth soils produced wheats with higher thiamine contents. An environmental effect other than that for soil zone on the thiamine and riboflavin content of wheat and oats was confirmed.

Recent reports have dealt with the thiamine and riboflavin contents of wheat, barley, and oats grown in Alberta (4), and Manitoba (6), and with the thiamine content of wheat grown in Saskatchewan (7). Attempts were made to assess the influence of soil zone and variety on vitamin content, and correlation coefficients were determined for each of the vitamins with other constituents of the grains. The reports for Alberta and Saskatchewan covered studies made with cereals grown in two successive crop years, that for Manitoba dealt with one year only, 1946. This paper presents results of a similar investigation of the 1947 Manitoba crop. More detailed reference to previous findings will be made later in this report.

Experimental

Approximately 120 samples of wheat, 80 of oats, and 50 of barley were obtained from Junior Seed Clubs and from the Laboratory of Cereal Breeding

[1] *Manuscript received in original form March 8, 1950, and, as revised, June 14, 1950. Contribution from the Department of Chemistry, University of Manitoba, Winnipeg, Manitoba, with financial assistance from the National Research Council of Canada. Published as Paper 280 of the Associate Committee on Grain Research.*

[2] *Associate Professor of Chemistry.*

[3] *Research Assistant.*

located on the University campus. Each sample was of a pure variety of grain. Variety was identified, as was the exact location where each sample was grown. This made it possible to identify the soil type. Included in the samples from the Laboratory of Cereal Breeding were several sets grown in the same soil zone, black earth, but at different locations in the province. These made possible a demonstration of the effect of environment other than soil zone.

Each sample was analyzed for moisture by the 130°C. oven method and other results were calculated to a 13.5% moisture level. Ash was determined by direct ignition in a muffle furnace and protein by the conventional Kjeldahl procedure. The methods for thiamine and riboflavin were reported previously (8).

Results and Discussion

Results of analyses, grouped according to variety and soil zone, are shown in Tables I–VI inclusive.

Barley

Thiamine contents of the barley samples tested showed a range of 3.3 to 5.7 μgm. per gm. The over-all average of 4.2 is not reported in the tables. Individual values are slightly higher than the corresponding ones for the 1946 crop,

TABLE I

THIAMINE CONTENTS OF BARLEY GROUPED ACCORDING TO VARIETY AND SOIL ZONE (μGM. PER GM.)

Variety	Soil zone						Averages for varieties
	Rendzina	Black earth	Dark-brown steppe–black earth transition	Northern black	Gray black	Gray wooded	
Garton		(2) 4.27					(2) 4.27
S.d.		0.01					0.01
OAC 21	(2) 4.48	(5) 4.49	(2) 3.60	(1) 3.71			(10) 4.23
S.d.	0.15	0.47	0.08	–			0.52
Sanalta	(1) 5.31	(7) 4.13	(2) 3.99	(1) 3.74			(11) 4.18
S.d.	–	0.18	0.18	–			0.41
Montcalm	(3) 4.81	(9) 4.48	(2) 4.23	(2) 3.59	(2) 3.83	(2) 3.65	(20) 4.27
S.d.	0.74	0.50	0.28	0.14	0.08	0.18	0.62
Mensury		(1) 4.35			(2) 4.03		(3) 4.13
S.d.		–			0.10		0.17
Plush		(2) 4.11		(4) 3.85			(6) 3.94
S.d.		0.80		0.39			0.58

Figures in parentheses in Tables I–VI inclusive are numbers of samples tested.

the range for which was 3.3. to 4.9 and the average 4.06 μgm. per gm. Varieties do not differ much. Even Garton and Mensury, for which there were available only two and three samples, respectively, show averages close to those for other varieties. Garton, OAC21, Sanalta, and Montcalm are about equal and slightly better than Plush. In 1946, Plush was better than OAC21. The lack of signi-

ficant difference between the varieties OAC21, Sanalta, and Montcalm was confirmed by an analysis of variance of the data for these varieties. This statistical study did not reveal any significant difference between thiamine contents of barleys grown on rendzina, black earth, brown black, or northern black soils. The lack of soil zone effect noted in the 1946 crop is confirmed for 1947. McElroy, Kastelic, and McCalla (4) found the thiamine contents of wheat, oats, and barley grown on brown soils to be significantly higher than those for the same cereals grown on gray ones. None of the Manitoba samples were grown on brown soils.

Riboflavin contents of the samples of barley tested ranged from 0.9 to 1.6 μgm. per gm. with an average of 1.3. This is almost identical with the 1946

TABLE II

RIBOFLAVIN CONTENTS OF BARLEY GROUPED ACCORDING TO VARIETY AND SOIL ZONE (μGM. PER GM.)

Variety	Soil zone						Averages for varieties
	Rendzina	Black earth	Dark brown steppe–black earth transition	Northern black	Gray black	Gray wooded	
Garton		(2) 1.42					(2) 1.42
S.d.		0.03					0.03
OAC 21	(2) 1.44	(5) 1.38	(2) 1.26	(1) 1.28			(10) 1.36
S.d.	0	0.12	0	–			0.10
Sanalta	(1) 1.20	(7) 1.42	(2) 1.28	(1) 1.12			(11) 1.35
S.d.	–	0.13	0.26	–			0.18
Montcalm	(3) 1.37	(9) 1.31	(2) 1.33	(2) 1.22	(2) 1.16	(2) 1.20	(20) 1.28
S.d.	0.08	0.09	0	0.07	0	0.02	0.10
Mensury		(1) 1.44			(2) 1.05		(3) 1.18
S.d.		–			0.16		0.22
Plush		(2) 1.29		(4) 1.08			(6) 1.15
S.d.		0.07		0.06			0.12

results, with a range of 0.9 to 1.9 and an average of 1.28 μgm. per gm. There is no varietal effect on riboflavin; this confirms the findings for 1946. Neither is there a soil zone effect. These last two observations were confirmed by an analysis of variance for OAC21, Sanalta, and Montcalm for rendzina, black earth, brown black, and northern black soil zones. This, too, is in agreement with observations for the previous year.

Oats

Thiamine in oats ranged from 3.8 to 8.6 μgm. per gm. with an average of 6.7. This is distinctly higher than for 1946, with a range of 4.4 to 7.9 and an average of 5.99 μgm. per gm. Analyses of variance were made of the data for Ajax and Vanguard grown on rendzina, black earth, and brown black soils, and for Exeter and Vanguard grown on rendzina, black earth, northern black, and gray wooded soils. These reveal neither varietal effect nor soil zone effect. Our 1946 observation, that Exeter is a better source of thiamine than either Ajax

TABLE III

THIAMINE CONTENTS OF OATS GROUPED ACCORDING TO VARIETY AND SOIL ZONE (μGM. PER GM.)

Variety	Soil zone					Averages for varieties
	Rendzina	Black earth	Dark brown steppe–black earth transition	Northern black	Gray wooded	
Ajax	(2) 5.15	(14) 6.78	(4) 6.27			(20) 6.51
S.d.	0.04	1.19	0.23			1.12
Exeter	(1) 6.70	(6) 7.74		(8) 6.26	(2) 6.08	(17) 6.78
S.d.	–	0.66		0.80	0.11	0.98
Vanguard	(1) 5.64	(13) 6.98	(1) 6.10	(5) 5.20	(1) 5.27	(21) 6.37
S.d.	–	0.76	–	0.60	–	1.05
Victory		(5) 7.88				(5) 7.88
S.d.		0.45				0.45
Garry		(5) 6.46				(5) 6.46
S.d.		0.27				0.27
Beacon		(5) 7.40				(5) 7.40
S.d.		0.15				0.15
Clinton		(5) 7.49				(5) 7.49
S.d.		0.36				0.36
Anthony				(1) 5.68		(1) 5.68
S.d.				–		–
Green Russian					(1) 5.33	(1) 5.33
S.d.					–	–

TABLE IV

RIBOFLAVIN CONTENTS OF OATS GROUPED ACCORDING TO VARIETY AND SOIL ZONE (μGM. PER GM.)

Variety	Soil zone					Averages for varieties
	Rendzina	Black earth	Dark brown steppe–black earth transition	Northern black	Gray wooded	
Ajax	(2) 1.19	(14) 1.31	(4) 1.06			(20) 1.25
S.d.	0.04	0.16	0.11			0.17
Exeter	(1) 1.43	(6) 1.49		(8) 1.35	(2) 1.31	(17) 1.40
S.d.	–	0.17		0.09	0.01	0.14
Vanguard	(1) 1.16	(13) 1.37	(1) 1.31	(5) 1.28	(1) 1.38	(21) 1.33
S.d.	–	0.07	–	0.03	–	0.08
Victory		(5) 1.48				(5) 1.48
S.d.		0.16				0.16
Garry		(5) 1.41				(5) 1.41
S.d.		0.12				0.12
Beacon		(5) 1.24				(5) 1.24
S.d.		0.12				0.12
Clinton		(5) 1.34				(5) 1.34
S.d.		0.08				0.08
Anthony				(1) 1.25		(1) 1.25
S.d.				–		–
Green Russian					(1) 1.25	(1) 1.25
S.d.					–	–

or Vanguard, is not confirmed. The one that soil zone is not a contributing factor to the thiamine content of oats is supported.

Riboflavin contents of oats ranged from 1.0 to 1.7 μgm. per gm. with an average of 1.3. The 1946 range was 0.8 to 1.7 with an average of 1.25. The data of Table IV do not reveal either varietal effect or soil zone effect on the riboflavin content of oats. These observations were confirmed by two analyses of variance for riboflavin, for the same samples and soil zones as for thiamine. In neither case was the F value for varieties as large as that for the 5% point. No soil zone effect on riboflavin content could be detected. Both of these observations confirm findings for the previous year.

Wheat

The thiamine content of wheat samples tested ranged from 3.4 to 5.9 μgm. per gm. with an average of 4.5. For 1946 the range was 3.8 to 5.7 and the average 4.28 μgm. per gm. The data are grouped according to variety and soil zone in Table V.

There is a definite varietal effect. The order of thiamine contents—for those varieties of which more than 10 samples were tested—is Carlton, Regent, Redman, Thatcher. This is the same order as obtained for 1946, save for Redman

TABLE V

THIAMINE CONTENTS OF WHEAT GROUPED ACCORDING TO VARIETY AND SOIL ZONE (μGM. PER GM.)

Variety	Soil zone						Averages for varieties
	Rendzina	Black earth	Dark brown steppe–black earth transition	Northern black	Gray black	Gray wooded	
Stewart		(4) 5.47					(4) 5.47
S.d.		0.16					0.16
Mindum		(4) 5.33					(4) 5.33
S.d.		0.23					0.23
Pelissier		(4) 5.19					(4) 5.19
S.d.		0.32					0.32
Carlton		(7) 5.26	(2) 4.22			(1) 4.63	(10) 4.99
S.d.		0.53	0.40			–	0.64
Red Bobs		(5) 4.92					(5) 4.92
S.d.		0.51					0.51
Renown	(1) 4.32	(1) 4.96					(2) 4.64
S.d.	–	–					0.32
Regent	(2) 4.77	(15) 4.81	(2) 4.44	(5) 3.88	(1) 3.91	(1) 3.92	(26) 4.53
S.d.	0.18	0.29	0.13	0.12	–	–	0.49
Marquis		(5) 4.48					(5) 4.48
S.d.		0.51					0.51
Rescue		(5) 4.31					(5) 4.31
S.d.		0.35					0.35
Redman	(1) 4.56	(18) 4.38	(5) 4.31	(7) 4.15		(5) 4.12	(36) 4.30
S.d.	–	0.38	0.41	0.16		0.28	0.35
Thatcher	(2) 4.49	(6) 4.05		(5) 3.85			(13) 4.04
S.d.	0.18	0.29		0.28			0.34
Apex		(5) 3.95					(5) 3.95
S.d.		0.26					0.26

which was not tested that year. These data confirm the findings of Jackson and Whiteside (2) and Whiteside and Jackson (9) who found the thiamine content of Regent wheat to be usually higher than those of Red Bobs, Thatcher, or Marquis. Separate analyses of variance were made for Regent, Thatcher, and Redman, and for Carlton, Regent, and Redman. In each case the F values for varieties was above the 5% point. Of the other varieties for which data are available, Stewart, Mindum, and Pelissier are the best and rank with Carlton. Red Bobs is about the same as Regent, Marquis and Rescue the same as Redman, and Apex on a par with Thatcher. Durum wheats are better sources of thiamine than hard spring wheats. Spencer and Galgan (7) found Stewart wheat to contain significantly greater amounts of thiamine than Thatcher, Rescue, or Pelissier.

The data reveal that wheats grown on rendzina and black earth soils had higher thiamine contents than the others. In 1946 no soil zone effect was observed. This 1947 soil zone effect on thiamine content was confirmed by analyses of variance. An F value for soil zones in excess of the 5% point was obtained in analyses of Regent and Redman grown on rendzina, black earth, brown black, northern black, and gray wooded soils, and of Regent, Thatcher, and Redman grown on rendzina, black earth, and northern black. An F value

TABLE VI

RIBOFLAVIN CONTENTS OF WHEAT GROUPED ACCORDING TO VARIETY AND SOIL ZONE (μGM. PER GM.)

Variety	Soil zone						Averages for varieties
	Rendzina	Black earth	Dark brown steppe–black earth transition	Northern black	Gray black	Gray wooded	
Stewart		(4) 1.13					(4) 1.13
S.d.		0.05					0.05
Mindum		(4) 1.21					(4) 1.21
S.d.		0.06					0.06
Pelissier		(4) 1.17					(4) 1.17
S.d.		0.06					0.06
Carlton		(7) 1.10	(2) 1.23			(1) 1.28	(10) 1.14
S.d.		0.03	0.04			–	0.07
Red Bobs		(5) 1.18					(5) 1.18
S.d.		0.12					0.12
Renown	(1) 1.09	(1) 1.27					(2) 1.18
S.d.	–	–					0.09
Regent	(2) 1.25	(15) 1.16	(2) 1.18	(5) 1.06	(1) 1.02	(1) 1.10	(26) 1.14
S.d.	0.09	0.05	0.14	0.04	–	–	0.08
Marquis		(5) 1.26					(5) 1.26
S.d.		0.12					0.12
Rescue		(5) 1.17					(5) 1.17
S.d.		0.09					0.09
Redman	(1) 0.97	(18) 1.15	(5) 1.16	(7) 1.06		(5) 1.25	(36) 1.14
S.d.	–	0.09	0.07	0.03		0.09	0.10
Thatcher	(2) 1.21	(6) 1.23		(5) 1.13			(13) 1.19
S.d.	0.05	0.08		0.05			0.08
Apex		(5) 1.22					(5) 1.22
S.d.		0.10					0.10

below the 5% point was obtained for Carlton, Regent, and Redman grown on black earth, brown black, and gray wooded soils. Johannson and Rich (3) found no relationship between soil type and thiamine content of commercial samples of wheat. The survey for Saskatchewan (7) did not reveal any soil zone effect either. However, McElroy, Kastelic, and McCalla (4) found that wheat grown on brown soils contained more thiamine than wheat grown on gray ones.

There does not seem to be either soil zone or varietal effect on riboflavin content of wheat. This is confirmed by analyses of variance. This confirms our observations for 1946. It is contrary to the results reported by Andrews, Boyd, and Terry (1), who listed wheat varieties tested, in order of decreasing riboflavin contents, as Marquis, Thatcher, Renown. Our 1947 values for ribo-

TABLE VII

THIAMINE CONTENTS OF WHEAT AND OATS GROUPED ACCORDING TO VARIETY AND STATION (μGM. PER GM.)

Variety	Station						
	Winnipeg	Portage la Prairie	Gilbert Plains	Morden	Brandon	Melita	Mean of all stations
Spring wheats							
Red Bobs	5.7	5.2	4.9	4.7	4.2		4.9
Marquis	5.3	4.8	4.4	4.4	3.7		4.5
Redman	5.1	4.4	4.2	4.0	3.9		4.3
Rescue	5.0	4.2	4.1	4.3	4.0		4.3
Thatcher	4.7	4.0	4.0	3.8	3.8		4.1
Apex	4.4	4.0	3.7	4.0	3.7		4.0
Mean of all varieties	5.0	4.4	4.2	4.2	3.9		
Durum wheats							
Carlton	5.9			5.8	5.5	5.4	5.7
Stewart	5.7			5.5	5.3	5.4	5.5
Mindum	5.7			5.3	5.0	5.4	5.4
Pelissier	5.4			5.4	5.1	4.7	5.2
Mean of all varieties	5.7			5.5	5.2	5.2	
Oats							
Exeter	8.6	7.6	7.4	8.9	7.6		8.0
Victory	8.4	8.0	7.4	8.3	7.3		7.9
Vanguard	7.8	8.1	7.5	7.8	7.5		7.7
Clinton	7.7	7.0	7.9	7.8	7.1		7.5
Ajax	7.9	7.5	7.2	7.6	6.6		7.4
Beacon	7.3	7.6	7.5	7.4	7.2		7.4
Garry	6.3	6.6	6.7	6.8	6.0		6.5
Mean of all varieties	7.7	7.5	7.4	7.8	7.0		

These data are for single samples grown on black earth soils.

flavin in wheat ranged from 1.0 to 1.4 μgm. per gm. with an average of 1.2. In 1946 the range was 0.8 to 1.4 and the average 1.12 μgm. per gm.

Effect of Environment

Samples of spring wheat, durum wheat, and oats obtained from the Laboratory of Cereal Breeding included several sets of varieties grown at each of four or five different stations throughout the province. All of these were grown on black earth soils. The thiamine and riboflavin contents of these are reported in Tables VII and VIII, respectively. Each table is in three parts to show the effect of location on vitamin content of each of spring wheats, durum wheats, and oats. The locations of stations were not all the same for durum wheats as for the other two.

TABLE VIII

RIBOFLAVIN CONTENTS OF WHEAT AND OATS GROUPED ACCORDING TO VARIETY AND STATION (μGM. PER GM.)

Variety	Station						
	Winnipeg	Portage la Prairie	Gilbert Plains	Morden	Brandon	Melita	Mean of all stations
Spring wheats							
Red Bobs	1.4	1.2	1.1	1.0	1.1		1.2
Marquis	1.4	1.4	1.1	1.3	1.2		1.3
Redman	1.4	1.2	1.2	1.2	1.2		1.2
Rescue	1.3	1.2	1.1	1.0	1.2		1.2
Thatcher	1.3	1.3	1.2	1.1	1.3		1.2
Apex	1.4	1.2	1.2	1.2	1.1		1.2
Mean of all varieties	1.4	1.3	1.2	1.1	1.2		
Durum wheats							
Carlton	1.1			1.1	1.1	1.1	1.1
Stewart	1.2			1.1	1.1	1.1	1.1
Mindum	1.3			1.3	1.2	1.2	1.3
Pelissier	1.3			1.2	1.1	1.1	1.2
Mean of all varieties	1.2			1.2	1.1	1.1	
Oats							
Exeter	1.7	1.5	1.7	1.5	1.3		1.5
Victory	1.7	1.3	1.5	1.6	1.3		1.5
Vanguard	1.5	1.3	1.5	1.4	1.3		1.4
Clinton	1.3	1.3	1.5	1.3	1.3		1.3
Ajax	1.4	1.1	1.3	1.3	1.0		1.2
Beacon	1.3	1.2	1.4	1.2	1.1		1.2
Garry	1.6	1.4	1.2	1.5	1.4		1.4
Mean of all varieties	1.5	1.3	1.4	1.4	1.2		

These data are for single samples grown on black earth soils.

The data of Table VII confirm that there is an effect of environment excluding soil on the thiamine content of these cereals. Other investigators (5, 9) have reported an environmental effect, but in their studies environment included soil zone. Winnipeg spring wheats were highest in content of this vitamin in every instance. Brandon ones were lowest in all cases but one. Portage la Prairie spring wheats usually placed second, while Morden and Gilbert Plains ones were about equal. This environmental effect is the same for durum wheats, except that the new station, Melita, usually showed lower values than Brandon —there were no samples from either Portage la Prairie or Gilbert Plains. The effect of environment on the thiamine content of oats was not as marked. The decreasing order of mean values was Morden, Winnipeg, Portage la Prairie, Gilbert Plains, and Brandon. This environmental effect was confirmed by analyses of variance. In every case the F value for stations was greater than the 5% point.

The range from the lowest to the highest riboflavin content is too small to show decided contrast between samples from different stations. The data of Table VIII show that Winnipeg-grown samples are usually higher in riboflavin content than the samples from elsewhere. It is more obvious for wheat than for oats. That there is an environmental effect, however, is revealed by the analysis of variance of these data.

Correlation Coefficients

Correlation coefficients were calculated for each of thiamine and riboflavin with each other, and with each of protein and ash. These coefficients are re-

TABLE IX

CORRELATION COEFFICIENTS FOR THIAMINE AND RIBOFLAVIN WITH PROTEIN AND ASH

Cereal	Barley	Oats	Wheat
Number of samples	55	80	122
Protein–thiamine	.4629**	.5938**	.3814**
Protein–riboflavin	.1333	.2455*	.0422
Ash–thiamine	.3269*	.1739	.2643**
Ash–riboflavin	−.1212	.3855**	.1511
Thiamine–riboflavin	.2115	.4401**	.0099

* *Within 5% level of significance.*
** *Within 1% level of significance.*

produced in Table IX. Marked positive correlations were found for protein and thiamine in each of the three cereals, and for protein and riboflavin in oats. Positive correlations were found for ash and thiamine in barley and wheat, and for ash and riboflavin in oats. There was also a correlation between thiamine and riboflavin in oats. Statistical studies of the data for the cereals grown in Manitoba in 1946 showed significant positive correlations between protein and thiamine in oats and barley, protein and riboflavin in wheat, and ash and ribo-

flavin in barley. A significant negative correlation between ash and thiamine in oats was also observed in 1946. There is thus no consistency in the correlation coefficients for the two years. This same lack of consistency is evident from other observations. Johannson and Rich (3) found no correlation between thiamine and either protein or ash in wheat, whereas McElroy *et al.* (4) found one for protein and thiamine in wheat, but none for protein and thiamine in barley. Spencer and Galgan (7) found a significant positive correlation between thiamine and protein content of wheat. The lack of correlation for ash and riboflavin in wheat confirms the findings of Nordgren and Andrews (5). McElroy *et al.* obtained no correlation between riboflavin and protein in barley and oats, which was the same as our finding, but on the other hand they did not find any between riboflavin and protein in wheat either.

Acknowledgments

Grateful acknowledgment is made to Mr. Whiteman and the District Representatives of the Extension Service of the Manitoba Department of Agriculture and to the Dominion Laboratory of Cereal Breeding for the collection of samples; to Prof. J. H. Ellis of the Department of Soils, University of Manitoba, who identified the soil zones where the samples were grown; and to Mr. Langtry E. Lynd for technical assistance.

References

1. Andrews, J. S., Boyd, H. M., and Terry, D. E. Cereal Chem. 19: 55-63. 1942.
2. Jackson, S. H. and Whiteside, A. G. O. Sci. Agr. 22: 366-377. 1942.
3. Johannson, H. and Rich, C. E. Cereal Chem. 19: 308-313. 1942.
4. McElroy, L. W., Kastelic, J., and McCalla, A. G. Can. J. Research, F, 26: 191-200. 1948.
5. Nordgren, R. and Andrews, J. S. Cereal Chem. 18: 802-811. 1941.
6. Robinson, Allen D., Lynd, Langtry E., and Miles, Barbara. Can. J. Research, F, 27: 241-248. 1949.
7. Spencer, E. Y. and Galgan, M. W. Can. J. Research, F, 27: 450-456 1949.
8. Spencer, E. Y., Robinson, A. D., McElroy, L. W., and Kastelic, J. Can. J. Research, F, 27: 194-198. 1949.
9. Whiteside, A. G. O. and Jackson, S. H. Cereal Chem. 20: 542-551. 1943.

THE EMULSION POLYMERIZATION OF ISOPRENE[1]

By R. H. Clark, Harold MacLean, Neil Henderson, George M. Barton, Arthur L. Johnson, Jean T. Grunlund, and Warren L. Reynolds

Abstract

The polymerization of isoprene has been initiated by peroxide–peroxidase, by various inorganic and organic catalysts, and by air oxidation and anodic oxidation. At moderately high conversion, polymers of isoprene have shown zero gel content and a dilute solution viscosity from 1.5 to 4. The physical properties of some of the polymers have been measured either in our laboratories or those of the Polymer Corporation of Canada.

Introduction

Ambros (1) has reported that the serum from freshly coagulated natural rubber latex, in the presence of an emulsifying agent and hydrogen peroxide, polymerized isoprene to a rubber-like material.

Although Ambros concluded from his results that the catalase–oxidase enzyme combination was the essential impetus for the polymerization, we feel that the results leave this point in doubt. It would appear to us that the necessity for hydrogen peroxide suggests the action of peroxidase. The latter enzyme acts on its substrate, hydrogen peroxide, to provide a nascent oxygen, whereas catalase merely decomposes hydrogen peroxide to water and molecular oxygen. It was decided, therefore, to attempt the polymerization of isoprene by plant peroxidase and hydrogen peroxide.

Experimental

Peroxidase was obtained from wild horseradish roots by the method of Elliott (6, 7, 10). It had a P.Z. (13) number of 300 which compared favorably with the value obtained by Elliott.

The polymerization recipe employed consisted of:

Water	180 gm.
Isoprene	60 gm.
R.R.C. soap	5 gm.
Hydrogen peroxide	2 ml. of 3% solution
Peroxidase enzyme	1000 peroxidase units

Agitation was provided by an end over end motion of the bottles, 18 times per minute, in a thermostat maintained at 35°C. The yields were measured by determining the total solids, with an allowance for the other solids present. Coagulation was effected with a brine-acid solution containing 367 gm. of

[1] *Manuscript received in original form July 29, 1949, and, as revised, May 23, 1950.*
Contribution from the Department of Chemistry, University of British Columbia, Vancouver, B.C.

sodium chloride and 10.5 cc. sulphuric acid in 6 liters of water. The crumb was washed in hot water and dried in a vacuum. Gel and intrinsic viscosity were measured by the conventional methods (4, 11). Yields were exceedingly erratic. Table I gives some idea of the variations.

TABLE I

POLYMERIZATION INITIATED BY PEROXIDASE AND HYDROGEN PEROXIDE

Polymerization time, hr.	Minimum yield, %	Maximum yield, %
18	5	35
40	30	53
48	32	56

A one-kilogram sample of the wet crumb from enzyme polymerization was forwarded to the Polymer Research and Development Division of the Polymer Corporation for compounding and testing. Table XI shows a comparison of this polyisoprene, Sample I, with natural rubber and GR–S.

The rate of cure of the polyisoprene sample was found to be notably faster than that of natural or GR–S rubber in an equivalent formulation. Sample I as received at Sarnia contained 78% gel. It compounded very poorly, was dry and crumbly, and did not band well on the mill.

The Polymer Research and Development Division suggested the use of short stoppers (H.Q.), and stabilizers (P.B.N.A.) for the protection of the finished product and the use of modifiers (D.D.M., DIXIE, carbon tetrachloride) during polymerization to control gel formation.

Each of these suggested modifiers, however, was found to inhibit the reaction with peroxidase.

The literature indicated that a trace of ferrous iron in the presence of hydrogen peroxide acts in a manner analogous to that of peroxidase (12). The iron salt was substituted for the peroxidase.

Preliminary experiments showed the following formula to be satisfactory, and all subsequent experiments were conducted at 45°C., using this recipe unless otherwise indicated.

Water	225 gm.
Isoprene	100 gm.
R.R.C. soap	4.2 gm.
H_2O_2	6 ml. of 3% solution
$FeSO_4$	0.1 gm.
D.D.M.	0.18 gm.

Note: List of abbreviations used.

R.R.C. — Rubber Reserve Corporation
D.D.M. — Dodecylmercaptan
Dixie — Diisopropyl-xanthogen disulphide
P.B.N.A. — Phenyl-beta-naphthylamine
H.Q. — Hydroquinone
M.T.M.4 — Mixed tertiary mercaptan

The bottles were charged by dissolving the soap and ferrous sulphate in hot water. After cooling the isoprene, D.D.M. and hydrogen peroxide were added just prior to capping.

In all our experiments the gel point set in very suddenly over a narrow increase of conversion, as is shown in Table II.

In each table, with the exception of I and XI, the values recorded are representative of a number of experiments.

TABLE II

POLYMERIZATION INITIATED BY DODECYLMERCAPTAN, FERROUS SULPHATE, AND HYDROGEN PEROXIDE

Polymerization time, hr.	Conversion, %	Gel content (11)	Intrinsic (4) viscosity
17	65.5	0	2.14
18	66.5	0	2.20
19	70	0	2.39
20	73.5	0	2.61
20 1/2	78	0	2.65
21	86	67	—

The reactions were stopped by the addition of 3 ml. of a saturated solution of hydroquinone. P.B.N.A. was used as an antioxidant, 1.25% on dry polymer weight.

It was observed that the length of time that elapsed between the mixing and using of the soap – ferrous sulphate combination gave proportionally higher yields as time increased. Consequently, two identical sets of charges were

TABLE III

THE EFFECT OF AN AGING PERIOD

Aging period, hr.	%Conversion in fixed time		Gel content		Intrinsic viscosity		Polymerization time, hr.
	Air excluded	Normal	Air excluded	Normal	Air excluded	Normal	
0	69	75	0	0	2.31	2.76	20
8	68	82	0	0	2.30	2.77	20
24	70	83.5	0	0	2.41	2.79	20
72	75	67.5	0	0	1.90	1.67	20

prepared for polymerization, with one set having air excluded and the other set charged without such precautions. The contrast is shown in Table III.

From these data it may be seen that a certain amount of air oxidation increases both yield and viscosity without raising the gel content. Excess oxygen as shown in the 72 hr. aging period reduces both yield and viscosity.

Air oxidation and an additional charge of hydrogen peroxide should have much the same effect. The results tabulated in Table IV confirm this view.

TABLE IV

THE EFFECT OF VARYING THE CONCENTRATION OF THE HYDROGEN PEROXIDE

H_2O_2	Conversion 20 hr., %	Gel	Intrinsic viscosity
10 ml. 3% solution	51	0	2.04
20 ml. 3% "	72	28	2.70
3 ml. 30% "	54	0	1.85
4 ml. 30% "	32	0	—
5 ml. 30% "	16	0	—
10 ml. 30% "	9	0	—

The aging period and a small excess of hydrogen peroxide produce the same effect. Dixie inhibited the reaction completely, as did also the substitution of Dresinate 731, a rosin soap, for the R.R.C. soap. The aging period, we believed at first, was producing its effect on the polymerization rate through its action on the R.R.C. soap, forming peroxides. Our tests, however, failed to show any increase in the amount of peroxides present due to the aging period. The effect of the aging period is probably due to the absorption by the ferrous sulphate – soap solution of oxygen from the air, which later acts in the same manner as a slight excess of hydrogen peroxide. Both act on the mercaptan forming free radicals which initiate polymerization. A large excess of hydrogen peroxide acts on most of the mercaptan to produce the disulphide, which does not initiate polymerization.

It was found that the same effects were produced by adding the D.D.M. either at the beginning of the 20 hour aging period or at the end of this period. In the subsequent anodic and air oxidation procedures the D.D.M. was added at the beginning of the aging period. A second sample, Sample II, of polyisoprene prepared by the hydrogen peroxide–ferrous sulphate method, of intrinsic viscosity 2.5 and zero gel content, was sent to the Polymer Corporation for evaluation. The results are tabulated in Table XI.

The results tabulated in Tables III and IV show that air and hydrogen peroxide have much the same effect. As a consequence, we tried air oxidation alone using the following proportions:

Water	190 gm.
Isoprene	100 gm.
R.R.C. soap	4.2 gm.
D.D.M.	0.18 gm.
$FeSO_4$	0.1 gm.

The soap was added to 180 gm. of water at 100°C. and after solution was complete, the temperature was reduced to 28°C. Then the ferrous sulphate in 10 gm. of water and the D.D.M. were added to the cooled soap solution. Air was bubbled through the soap solution for 10 min. The aerated solution was set aside for 25 hr. or other chosen periods of time, and the isoprene, freshly distilled, was added just prior to placing in the polymerizer at 45°C.

Table V gives typical results for the air oxidation procedure.

TABLE V

THE EFFECT OF AN AGING PERIOD ON POLYMERIZATION INITIATED BY DODECYLMERCAPTAN AND AIR

Aging time, hr.	% Yield	Intrinsic viscosity	% Gel	Polymerization time, hr.
0	22	1.7	3.5	24
20 1/2	59	2.3	1.3	24
27 1/2	56.5	3.2	2.6	24
43 1/2	63.5	4.4	4.3	24

An activator solution (5) of 1 gm. of glucose and 0.6 gm. of sodium pyrophosphate was added to the Air Oxidation formula. The activator solution was added at different times during the aging period. Some typical results are given in Table VI.

TABLE VI

THE EFFECT OF ADDING AN ACTIVATOR SOLUTION TO THE AIR OXIDATION FORMULA

Time of adding the activator solution	Yield, %
At the beginning of the aging period	16
After 20 hr. of an aging period	31
At the end of the aging period	30
After 15 min. of polymerization	34
The aging period was 24 hr.	—
The polymerization time was 24 hr.	—

Polymerization rates are usually increased by, or at worst are indifferent to, a glucose activator solution. The decreased rate found here can be attributed to the reducing effect of the glucose on the oxidation products formed during the

aging period. It is apparent that the later the glucose is added, the closer the yield approaches that for polymerization without the activator, as given in Table V.

The results also supply further evidence that peroxides are not formed during the aging period. Polymerization with peroxides, e.g., cumene hydroperoxide, benzoyl peroxide, etc., is usually activated by glucose, instead of inhibited to the degree found here.

The activator solution was tried in a cumene hydroperoxide formula (9) of the following composition:

Water	195 parts by weight
R.R.C. soap	4.2 " " "
Glucose	1.0 " " "
$FeSO_4.7H_2O$	0.1 " " "
$Na_4P_2O_7.10H_2O$	1.0 " " "
Cumene hydroperoxide	0.17 " " "
M.T.M.4	0.45 " " "
Isoprene	100 " " "

Table VII gives the results obtained.

TABLE VII

POLYMERIZATION INITIATED BY CUMENE HYDROPEROXIDE

Polymerization time, hr.	Yield, %
2.5	47
3.5	56
4.5	60
4.75	65
5.0	78
7.5	92

The gel content, as determined for the higher yields, was unsatisfactory; up to 60% yield the gel content was zero. Evaluation of the vulcanizates made from polyisoprene produced by air oxidation was not made. However, very probably their evaluation would be similar to those for Sample II.

According to Brockman (2), electrolytic oxidation and oxidation by peroxides are similar in nature. This is further substantiated by Churchill (3), who examined the formation of hydrogen peroxide in electrolytic corrosion. Also Heinrich and Klemenc (8) showed that hydrogen peroxide was produced electrolytically at the anode. This suggested the use of anodic oxidation; two methods were employed.

(*a*) *Continuous*:—A steady flow of d-c. electricity was passed through an emulsion of isoprene at atmospheric pressure and 34°C.

(*b*) *Discontinuous*:—After a short treatment of the emulsion with d-c. electricity, the emulsion was transferred to 16 oz. bottles, sealed and placed in the polymerizer for specified periods.

1. *Continuous Method*:—The emulsion was comprised of either of the following:

(*a*) 90 cc. water (distilled)
2.5 gm. R.R.C. soap
30 gm. isoprene

(*b*) 90 cc. 0.01 *N* sodium hydroxide
2.5 gm. R.R.C. soap
30 gm. isoprene

It was placed in a 500 cc. three-necked flask fitted with a rotating platinum anode, a stationary cathode, and a reflux condenser. An external electric heater was used to maintain the temperature of boiling isoprene (i.e., 34°C.). The electrolytic current was varied from 90 to 118 ma., at a voltage range of 30 to 80. In periods of from three to six hours only low molecular weight oils were formed.

2. *Discontinuous Method*:—The emulsion was made up according to the following formula:

180 cc. water (distilled)
4.2 gm. R.R.C. soap
100 mgm. anhydrous ferrous sulphate
0.18 gm. D.D.M.
100 gm. isoprene (freshly distilled)

The soap and then the ferrous sulphate were dissolved in the boiled water. After cooling the mixture, the D.D.M. was added, and the current was passed through the mixture for the specified time. The isoprene was then added, and the mixture placed in the polymerizer at 45°C. for 24 hr. After polymerization had proceeded for that period, the reaction was short stopped, and the solution coagulated as before.

Results

TABLE VIII

THE EFFECT OF CURRENT DENSITY ON THE POLYMERIZATION OF ISOPRENE BY ANODIC OXIDATION

Polymerization time—24 hr. at 45°C.

Current, ma.	Time of current passage, min.	Area of Pt anode, $cm.^2$	Current density, $ma./cm.^2$	Gel	Intrinsic viscosity	% Yield
1.0	10	19.6	0.0510	0	2.3	58
1.0	10	42.6	0.0235	0	2.2	59
1.0	10	170.0	0.0058	0	2.2	57

In Table IX it will be observed that the ferrous sulphate is essential to the operation of the formula.

TABLE IX

THE EFFECT OF FERROUS SULPHATE ON THE POLYMERIZATION OF ISOPRENE BY ANODIC OXIDATION

Current, ma.	Time of current passage, min.	Time of polymerization, hr.	$FeSO_4$	Results
0.45	15	24	Normal	Normal polymer
0.45	15	24	None	No polymer
0.45	15	40	None	No polymer

Apparently the current density within these ranges has no effect; only the total amount of current influences the reaction.

Results recorded in Table X show an upward trend for intrinsic viscosity with increasing number of coulombs, reaching a maximum at 0.75 coulomb and falling off beyond that current. This parallels the results obtained with

TABLE X

THE EFFECT OF TOTAL CURRENT ON THE POLYMERIZATION OF ISOPRENE BY ANODIC OXIDATION

Current, ma.	Time of current flow, min.	Coulombs	Time of polymerization, hr.	Gel in %	Intrinsic viscosity	% Yield
0	0	0	25	2.6	1.58	38
1.5	3	0.27	25	9.6	1.71	42
0.5	15	0.45	23	1.9	2.19	40
1.0	10	0.60	24	0	2.30	58
1.0	12	0.72	25	9.6	2.48	60
1.4	9	0.75	25	12.2	2.93	83
1.5	10	0.90	28	4.4	2.76	72
2.5	10	1.5	24	0.0	2.56	59

an excess of hydrogen peroxide as previously found. A sample of polyisoprene III prepared by anodic oxidation, in which the yield was 70% in 40 hr., the intrinsic viscosity 2.7 and with gel content low, was tested by the Polymer Corporation with the results shown in Table XI.

For these tests the following formula was used:

Polymer	100 parts
Black	42 "
Zinc oxide	6 "
Stearic acid	3.5 "
Pine tar	4 "
P.B.N.A.	1 "
Sulphur	2.75
Captax	0.75
Cure temperature	275°F.

TABLE XI

SLOW CURING TREAD STOCK RECIPE

Test	Polyisoprene Sample I	Polyisoprene Sample II	Polyisoprene Sample III	Natural rubber	Standard test formula G.R.S.
Maximum tensile, psi.	1800	2540	2420	3800	3400
Modulus at 300% extension, psi.	900	400	400	950	1100
Elongation at break, %	490	900	900	700	650
Rebound, %	64	58.4	59	63	54
Raw polymer Mooney	——	58	62	——	——
Flex cut growth per 5 kilocycles of flex, in.	0.532	——	——	0.084	0.140
1 Kilocycle of flex	——	0.019	0.020		
Hysteresis heat rise, °F.		177	178		
Cure time, min.		30	25		
For gum stock formulation					
Maximum tensile, psi.	290	——	——	2600	600
Modulus, psi.	145	——	——	280	150
Elongation, %	575	——	——	680	890

The results of the tests made by the Research and Development Division of Polymer Corporation, Sarnia, are tabulated in Table XI. The values for Samples I, II, and III are given in the first three columns respectively, and the corresponding values for natural rubber and Standard Test Formula GR-S in columns 4 and 5.

The Polymer Corporation indicated by letter that the polyisoprene Samples II and III differ from natural rubber mainly in cold-mill breakdown and tensile strength.

Conclusions

Elastomers of polyisoprene have been prepared with intrinsic viscosities from 1.5 up to 4, with low gel content, and with conversions up to 90% in six hours. It has been noted that the faster the conversion of isoprene into the polymer, the less tough the elastomer appears to be. This may mean a more rapid breakdown on the mill compared to the tougher samples already tested. It is suggested that some of the polyisoprene samples be vulcanized by different processes and their properties evaluated.

Acknowledgment

The authors are very grateful to the National Research Council of Canada for the financial assistance that has made this investigation possible. They also wish to thank the Research and Development Division of Polymer Corporation, Sarnia, for its valuable assistance.

References

1. AMBROS, OTTO. Revue génerale caoutchouc, No. 113. July-August, 1937.
2. BROCKMAN, C. J. Electro organic chemistry. John Wiley & Sons, Inc., New York. 1926.
3. CHURCHILL, J. R. Trans. Electrochem. Soc. 76: 77. 1939.

4. CRAGG, L. W., ROGERS, T. H., and HENDERSON, B. A. Evaluation of the intrinsic viscosity of GR–S in benzene, OSR–38. Aug. 20, 1946.
5. EASTHAM, A. M. Private communication, June 3, 1948.
6. ELLIOTT, K. A. C. Biochem. J. 26: 1281. 1932.
7. ELLIOTT, K. A. C. and KEILIN, D. Proc. Roy. Soc. (London), B, 114: 210. 1934.
8. HEINRICH, G., and KLEMENC, A. Z. physik. Chem. 184: 347. 1939.
9. HOUSTON, R. J., FOX, G. L., WEISS, H. J., and POW, E. S. Bulletin, B. F. Goodrich Company, Akron, Ohio. April 15, 1947.
10. KEILIN, D. and MANN, T. Proc. Roy. Soc. (London), B, 122: 119. 1937.
11. KOLTHOFF, I. M. and MEDALIA, A. I. Report LXXXI. Determination of gel in GR–S. University of Minnesota. Oct. 15, 1945.
12. WALDSCHMIDT-LEITZ, ERNST. Enzyme action and properties. Chapman and Hall, Limited, London. 1929.
13. WILLSTATTER, R. and STOLL, A. Ann. Chem. Phys. 416: 21. 1918.

NOTES

Leveling Bulb Control for Null Manometers*

One of the difficulties met with in the use of leveling bulbs and null manometers is that of controlling the flow of the mercury in such a manner as to be able to easily adjust the level exactly at the null point. The bulbs and manometers are generally connected either by means of rubber tubing, with a pinch clamp serving as a regulator for the mercury flow, or they may be connected with glass tubing through a stopcock.

Both of these systems have inherent disadvantages. Distortion of the rubber tubing by the closing of the pinch clamp causes fluctuations in the level of the mercury in the burette or manometer. Atmospheric pressure changes also affect the mercury level in the rubber connected apparatus, and this causes an appreciable loss of time in resetting, especially when working at low pressures. Although glass systems are free of the above trouble, they are quite likely to become greasy from the lubricant, picked up by the mercury passing through the stopcock. The stopcock is also quite likely to blow out at pressures greater than atmospheric.

The authors have designed a system of control that eliminates the above defects. It is simply a mild steel needle valve sealed to a leveling bulb by means of deKhotinsky cement. The diagram shows all the details of the different components of the valve, which is composed of four parts: stem and needle (*A*), bonnet (*B*), body (*C*), and plastic valve seat (*D*). The dimensions have not been included as they are not critical. The valve operates as a simple needle type, with the one special feature being the groove cut in the threaded part of the stem. This groove is to allow the mercury to flow from the reservoir past the stem threads to the valve seat.

Some of the dimensions of the original valves are given below as a guide in future fabrications. The stem was made 8½ in. long from 3/16 in. diameter rod. The needle had a 60° angle at the point and the threaded portion was 1 3/8 in. long with 32 threads to the inch. The groove began 1/8 in. from the base of the needle and was 1/16 in. by 1/16 in. by 1½ in. The bonnet was 1 5/8 in. long, with an inside diameter of ½ in. at the open end. The central part, which supported the stem, was threaded for a distance of 3/8 in. with No. 10-32 NF threads. Standard 1/8 in. pipe thread was used for screwing the bottom of the bonnet to the top of the body. The body was 1 in. long in each direction. The lucite insert was 3/8 in. diameter and 5/16 in. long and was press fitted. The valve outlet was 5/16 in. diameter. The small hole through the center of the lucite was 1/16 in. diameter.

* *Also issued as DRCL Report No. 51.*

When assembling the valve, the needle was heated and screwed down against the lucite to form a bearing surface in the plastic. A piece of glass tubing that fitted snugly into the top of the bonnet was blown to the bottom of the leveling bulb and the two components sealed together with deKhotinsky cement. To ensure a vacuum tight joint between the bonnet and body, the threads of each

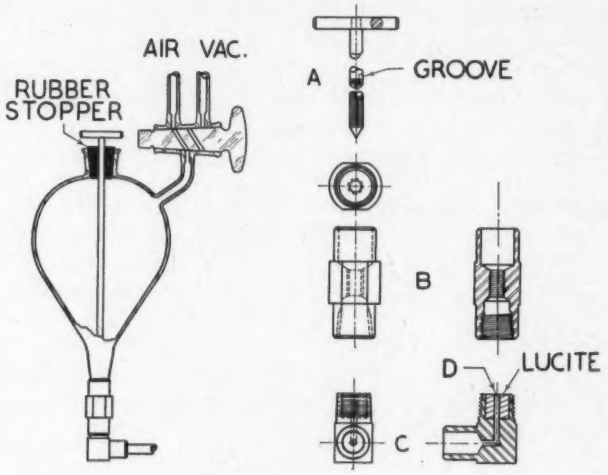

FIG. 1.

were warmed and coated with a low temperature cement, such as Plicene, and tightened before the cement set. The top of the leveling bulb was sealed by a tightly fitted rubber stopper around the stem. A little stopcock grease helped to make the bearing surface between the stem and the rubber reasonably vacuum tight. The bulb was connected to vacuum and pressure lines through a threeway stopcock and the level of the mercury changed by opening the needle with either a vacuum or pressure in the bulb.

RECEIVED JUNE 1, 1950.
DEFENCE RESEARCH CHEMICAL LABORATORIES,
OTTAWA, CANADA.

J. C. ARNELL
G. O. HENNEBERRY*

* *Present address: Macdonald College, Ste. Anne de Bellevue, Que.*